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PLAGUE ANTIPHAGE SERUM FROM RAMS

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We set ourselves the task of obtaining plague antiphage serum from rams. This serum actively suppresses phage.

The presence of phage in the organism frequently makes the isolation of plague cultures difficult and thus impedes diagnosis as well as the taking of prophylactic measures at the proper time.

A number of researchers has been concerned with the question of the suppression of phage and of freeing cultures from it. D'Herelle, to prevent the appearance of phenomena produced by bacteriophages, proposed to transplant the culture to agar-agar that contains glucose. Because of the growth of bacteria, the agar-agar gradually becomes acid, and the acid medium is unsuitable for the development of phage. Pozerskiy suggested transplanting a culture infected with phage to slanted agar-agar, then to carry out the transplantation several times, using material from the top portion of the slanted medium, because it is somewhat drier.

In 1939, Serebryakova obtained good results in experiments on the adsorption of phage by killed dysentery microbes. According to her data, the addition of killed microbes to the material under investigation offers better chances for establishing the presence of the microbe in the investigation.

According to Zhukov-Verezhnikov's and Favorisova's findings, bacteriophage and bacteria are not only simultaneously present in the organs of the experimental animals, but are transmitted together from guinea pig to guinea pig. This creates some difficulty as far as the direct isolation of plague cultures from the organs of guinea pigs is concerned, because the bacteriophage is seeded out into the medium together with the bacteria, and the conditions of the medium permit the bacteriophage to exert its influence. Also, according to Zhukov-Verezhnikov's and Favorisova's data, normal serum and the serum of a human being who had had pulmonary plague delay the process involved in the action of the bacteriophage on the plague microbe.

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For the suppression of dysentery bacteriophage in the feces of dysentery patients, Smirnov successfully used antiphage serum. According to his data, the antiphage serum lengthens the period during which dysentery bacteria can be seeded out.

Sinel'nikov, as the result of the immunization of sheep with polyvalent dysentery bacteriophage, succeeded in obtaining antiphage serum which neutralized 16 times the quantity of the original phage.

Mar'ina, by immunization of rabbits with various series of plague phage, obtained a serum which suppressed the bacteriophage satisfactorily. The serum which she obtained after three cycles of immunizations suppressed the phage much better than that obtained after only one cycle of immunization.

Up to the present, plague antiphage serum was obtained from rabbits. The use of rabbits for the mass production of antiphage serum is not economical, because in the immunization cycle less than 10 cc of serum are obtained from one rabbit, and besides, taking blood from the heart of the rabbit is difficult and requires a certain skill. All this impelled us to use rams as serum producers. It is known that among the large animals the ram is the most susceptible to plague and gives the most effective serum; furthermore, within one immunization cycle, it can supply as much as 350 cc of serum.

In our work, we began with the hyperimmunization of two rams with Berlin's plague phage. A total of ten intravenous injections was administered in an interval of 4-6 days. The dose of the first injection was 3 cc, that of the last injection 50 cc (cf. appended table).

On the 14th day, after the last injection of phage, the rams were bled twice within 24 hr.

Twenty-four hours after the blood was taken, the serum was drawn off and preserved with 0.5 percent of chloroform. Then the serum obtained was checked for sterility and titrated. We titrated the serum according to the following method: each serum was diluted to the concentrations 1:2, 1:4, 1:8, 1:16, 1:32 etc., with broth, and the phage was diluted to 1:100. Then 0.5 cc of each serum dilution were mixed with 0.5 cc of diluted phage. The mixture was left in a thermostat at 37° C for 2-3 hr. After this time, the mixture and the culture were brought together in an agar-agar dish; the mixture in a quantity of two drops, and the culture in a quantity which supplies a good "lawn." Then the dishes were again placed in the thermostat, and kept there at a temperature of 28° C, for 24 hr. The result of the titration was established at the expiration of 24 hr.

The serum obtained from ram No 1 completely suppressed the phage in a 1:16 dilution, while that of ram No 2 suppressed the phage in a dilution of 1:32. We also took the limit of dilution of the serum for each titer which completely suppressed the phage, i.e., left no sterile spots on the "lawn" of the culture.

On setting up a control with normal rams' serum, no suppression of phage was observed. Sterile tracks appeared in the dishes.

In simultaneous checking of the titer of plague antiphage serum obtained from rabbits by the same method of immunization, the titer of this serum was established at 1:2 and 1:4, which shows the undisputed superiority of rams' serum.

At present, the phage laboratory is making wide use of rams for the mass production of plague antiphage serum. In the second immunization cycle, the rams supply a much higher titer than in the first cycle (1:64 and above).

Thus, plague antiphage serum from rams can be recommended for wide use as a diagnostic preparation.

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Table 1. Berlin's Plague Phage (data for 1948, in cc)

Exptl Animal	Immunization Cycle	2/2	2/7	2/12	2/17	2/21	2/26	3/2	3/6	3/11	3/16	Rest in Days	Bloodletting I	Bloodletting II
Rams No 1 and 2	1	3	5	7	12	15	22	27	32	40	50	14	3/30	4/1

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